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Concentrations of Metals Associated with Mining Waste in Sediments, Biofilm, Benthic Macroinvertebrates, and Fish from the Coeur d'Alene River Basin, Idaho

A. M. Farag, D. F. Woodward, J. N. Goldstein, W. Brumbaugh, J. S. Meyer

U.S. Geological Survey, Environmental Contaminants and Research Center, Jackson Field Station, P.O. Box 1089, Jackson, Wyoming 83001, USA

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Abstract. Arsenic, Cd, Cu, Pb, Hg, and Zn were measured in sediments, biofilm, benthic macroinvertebrates, and fish from the Coeur d'Alene (CDA) River to characterize the pathway of metals transfer between these components. Metals enter the CDA Basin via tributaries where mining activities have occurred. In general, the ranking of food-web components from the greatest to smallest concentrations of metals was as follows: biofilm (the layer of abiotic and biotic material on rock surfaces) and sediments > invertebrates > whole fish. Elevated Pb was documented in invertebrates, and elevated Cd and Zn were documented in sediment and biofilm approximately 80 km downstream to the Spokane River. The accumulation of metals in invertebrates was dependent on functional feeding group and shredders-scrapers that feed on biofilm accumulated the largest concentrations of metals. Although the absolute concentrations of metals were the largest in biofilm and sediments, the metals have accumulated in fish approximately 50 km downstream from Kellogg, near the town of Harrison. While metals do not biomagnify between trophic levels, the metals in the CDA Basin are bioavailable and do biotransfer. Trout less than 100 mm long feed exclusively on small invertebrates, and small invertebrates accumulate greater concentrations of metals than large invertebrates. Therefore, early-lifestage fish may be exposed to a larger dose of metals than adults.

The Coeur d'Alene (CDA) River is located in northern Idaho and has received metals contamination from mining operations and smeltering since 1885 (Ellis 1940). Hornig *et al.* (1988), Holland *et al.* (1994), and Brennan *et al.* (1995) reported elevated concentrations of As, Cd, Cu, Hg, Pb, and Zn in the water, sediment, and benthic macroinvertebrates of the CDA River. The presence of metals has been associated with biological effects on aquatic and terrestrial organisms. For example, in 1991, species richness and Shannon-Weiner diversity indices of benthic invertebrates in the South Fork of the CDA River were less than those measured at reference sites. Moreover, these measurements were performed 23 years after impoundments were installed to limit the amount of metals entering the South Fork of the CDA River (Holland *et al.* 1994). In addition, trout populations in the South Fork of the CDA River are depressed (SAIC and EPT 1991) and Blus *et al.* (1991) documented lead toxicosis in waterfowl that consume river sediments in the CDA Basin.

While effects have been observed in areas of highest metal contamination in the CDA Basin, the pathway and partitioning of metals in the aquatic environment has not been well characterized. In metals-contaminated systems, the pathway of metals from contaminated sources to organisms in the system can be determined by measuring metal residues in the various components: water, sediments, biofilm, invertebrates, and fish from identical locations throughout a river system. These data can then be used to characterize the partitioning of abiotic contamination (water and sediment) and the transport of metals into the biotic community (biofilm, invertebrates, and fish).

Biofilm (often referred to as *aufwuchs*) consists of attached algae, bacteria, and associated fine detrital material that adheres to substrates in water bodies (Ruttner 1968) and is a food source for invertebrates that scrape mineral and organic surfaces (Merritt and Cummins 1978). Therefore, biofilm is a critical link between particle-bound metals of colloidal size, algae, and aquatic invertebrates with grazing-scraping feeding mechanisms (Kimball *et al.* 1995). Although Newman *et al.* (1983, 1985) documented that a portion of metals in procedurally defined *aufwuchs* is associated with abiotic components, the authors also measured metals in the algal component of biofilm. Thus, metals are associated with both the abiotic and biotic components of biofilm. Invertebrates consume both components when they feed on biofilm. Thus, biofilm samples are important for tracing the pathway of metals.

Benthic macroinvertebrates represent a concentrated source of metals that may be toxic in the diet of fish (Woodward *et al.* 1994, 1995). Factors that affect the uptake of metals in invertebrates can influence the distribution of metals in the CDA Basin. Benthic macroinvertebrates occupy various niches and functional feeding groups (Merritt and Cummins 1978) and their feeding behavior can determine the dose of metals received (Smock 1983; Timmermans *et al.* 1989; Kiffney and Clements 1993). Some collectors burrow into contaminated sediments and may feed on detrital materials contaminated with larger concentrations of metals than free-swimming prey often

Note: Use of trade names does not imply endorsement of a product.

Correspondence to: A. M. Farag

targeted by predators. If benthic macroinvertebrates differentially accumulate metals, then the concentrations of metals in composite samples of invertebrates will vary based on the representation of the various functional feeding groups.

Size can also influence the accumulation of metals in invertebrates. In fact, van Hattum *et al.* (1991) found that size and temporal variability had greater influences than sex or species on the accumulation of metals in isopods. Thus, small collectors may accumulate greater concentrations of metals than large collectors because of differences in ratios of body surface area to size. This difference is possible even though the two collectors feed at similar trophic levels. If body size affects the accumulation of metals in benthic macroinvertebrates, then juvenile fish that feed on smaller invertebrates will receive a greater dose of metals than adult fish that feed on larger invertebrates in the CDA River.

The objectives of this study were to (1) characterize the pathway of metals into water, sediments, biofilm (*aufwuchs*), invertebrates, and fish; and (2) to document the effect of functional feeding group and size on the accumulation of metals in benthic macroinvertebrates.

Methods

During the summer of 1994, sediments and biofilm were collected from 13 locations: 11 sites on the CDA River and its tributaries: North Fork (NF), Mullan (ML), Canyon Creek (CC), Nine Mile (NM), Pinehurst (PH), Cataldo (CT), Pine Creek (PC), Lower CDA River 1 (LCDR1), Lower CDA River 2 (LCDR2), Lower CDA River 3 (LCDR3), Harrison (HSR), one site on the Spokane River (SR), and one site on the St. Joe River (SJ) (Figure 1). NF, ML, and SJ were designated as reference sites. These three reference sites were chosen because there was little or no historical mining activity above these sites. Consequently, NF has been used as a reference site for other studies of the CDA River and concentrations of total recoverable metals were 0.05 µg Cd/L, 0.5 µg Pb/L, and 9.0 µg Zn/L in the water (Goldstein *et al.* in review). Concentrations of metals in invertebrates were reported as 0.97 µg Cd/g, 7.37 µg Pb/g, and 384 µg Zn/g (Farag *et al.* in review) from NF. Preliminary data collected by U.S. Fish and Wildlife Service personnel indicated that whole fish samples of cutthroat trout from SJ contained 0.224 µg Cd/g, 2.6 µg Pb/g, and 422 µg Zn/g. ML was added as a reference site following discussions of its appropriate riffle characteristics with U.S. Fish and Wildlife Service, Coeur d'Alene Tribe, and U.S. Forest Service personnel.

Benthic invertebrates were collected from all sites except SJ. Whole fish were collected from four sites on the CDA River (LCDR1, LCDR2, LCDR3, and HSR) and from SJ. Additionally, specific tissues were collected from fish from PH, CT, and NF, but due to an insufficient number of samples we were unable to measure metals in whole fish at PH and CT. Separately from this study, metal concentrations were measured monthly in water samples from the CDA River (Brennan *et al.* 1995; Harenberg *et al.* 1994).

Benthic macroinvertebrate samples were collected to determine the influence of functional feeding group and invertebrate size on the accumulation of metals. The functional feeding groups as defined by Merritt and Cummins (1978) are based on feeding mechanism: shredders, scrapers, collectors, engulfers, piercers, and parasites. The collectors are subdivided further into filterers or gatherers that have mouth parts developed for filtering suspended material or scavenging the sediment. The invertebrates were identified to genus, and thus specific details about the feeding mechanisms of each species cannot be determined. However, the classifications identified by Merritt and Cummins (1978) include the feeding behaviors of all of the possible species within a genus. Thus, we are able to compare these invertebrates based on differences in the general feeding mechanisms.

Invertebrates from more than one genus were composited in some cases to ensure that enough tissue was collected for accurate metal measurements. However, the composites consisted of genus representatives with similar feeding habits. Shredders-scrapers, *Pteronarcella* and *Pteronarcys* (Plecoptera:Pteronarcidae), shredders-collectors (gatherers) *Tipula* (Diptera:Tipulidae), collectors (filterers) *Arctopsyche* and *Hydropsyche* (Tricoptera:Hyrdopsychidae), and predaceous engulfers *Calineuria* (Plecoptera:Perlidae) were collected at CT. Piercers and parasites were not collected during our sampling effort. From this point, collectors (filterers) and collectors (gatherers) will simply be identified as filterers or gatherers for clarity. Large (*Arctopsyche*) and small (*Simulium* [Diptera:Simuliidae]) filterers were collected at PH. All samples were kept frozen until they were processed and analyzed for As, Cd, Hg, Pb, and Zn with atomic absorption spectroscopy.

The sites at NF, ML, CC, NM, PH, CT, PC, and SR were generally erosional environments with gravel riffles characteristic of high gradients. However, at LCDR1, LCDR2, LCDR3, HSR, and SJ the water was deeper, slower-moving, and more characteristic of depositional environments and low gradients. Slightly different sampling techniques were utilized in these two different environments and are described below. Each site consisted of between a 0.5- to 2-mile stretch of the river. The size of an individual site was dependent on the accessibility of replicate locations within that site. In general, the sites with high-gradient waters were slightly smaller than those with low-gradient waters.

Replicate Selection

In high-gradient waters, accessible riffles under at least 10 cm of water were identified within the boundaries of the site. Acceptable riffles were defined as those that covered a minimum area of approximately 6 m^2. (Note: If fewer than 10 acceptable riffles were identified within the boundaries of a site, larger riffles within that site were subdivided and designated as riffles of at least 6 m^2.) Four of these riffles were selected at random and were designated as the four sampling locations within the site. In low-gradient environments, 10 accessible areas along the shore with depths of 1 m or less were identified. Four of these areas were selected at random and identified as the four sampling locations.

Sediment Sampling Procedures

Sediments were collected with a plastic scoop from depositional areas in the high-gradient locations. When necessary, large rocks were moved so the fine sediment underneath could be collected. Any water present was decanted from the sample. Sediments were collected with a petite ponar at low-gradient sites. All tools were rinsed with deionized water and/or acid washed before being reused for additional collections.

Biofilm Sampling Procedures

Biofilm consists of abiotic and biotic material that adheres to rock surfaces in water bodies. Rocks along the shore were removed from the water, their surface was scraped gently with acid-washed plastic utensils, and the biofilm was placed directly into acid-washed plastic vials.

Benthos Sampling Procedure

In high-gradient locations, benthos were sampled with a 3-mm mesh net attached to a 1.2 m $\times$ 0.6 m frame. The substrate in approximately 6

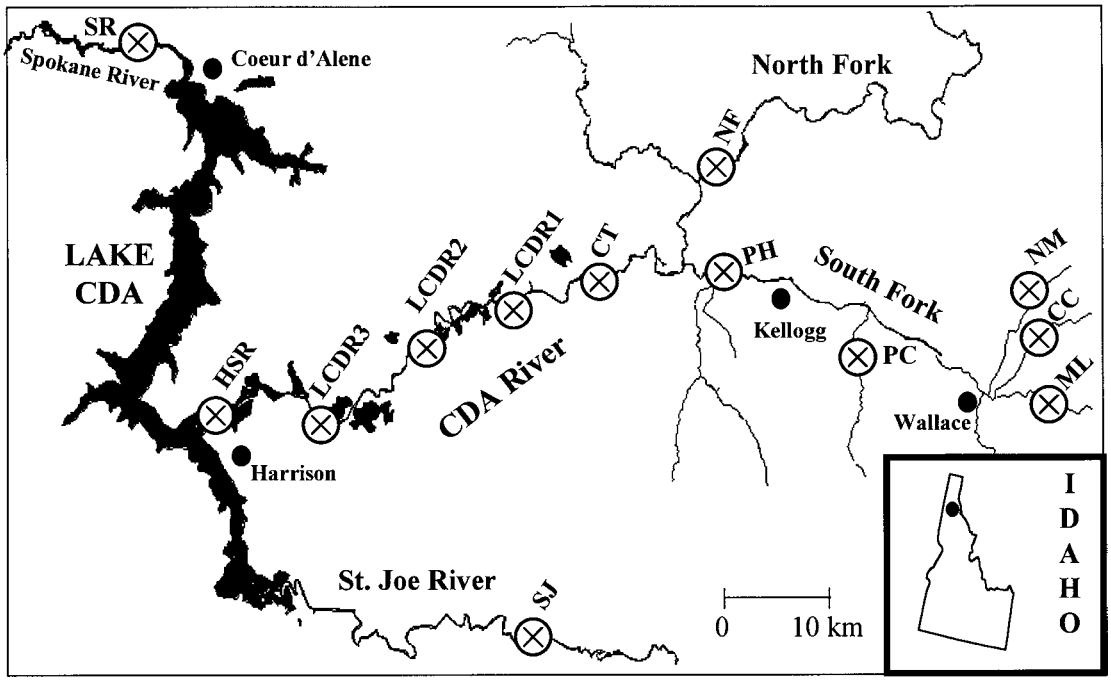

Fig. 1. Map of the Coeur d'Alene Basin in northern Idaho. The collection sites are designated with capitol letters: NF = North Fork, CC = Canyon Creek, CT = Cataldo, HSR = Harrison, LCDR1 = Lower CDR1, LCDR2 = Lower CDR2, LCDR3 = Lower CDR3, ML = Mullan, NM = Nine Mile, PC = Pine Creek, PH = Pinehurst, SJ = Saint Joe, and SR = Spokane River

m^2 of riffle above the net was overturned with hooked garden tools and the dislodged benthos were collected in the net. Benthos were removed from the net with acid-washed plastic forceps and placed into acid-washed plastic vials. In low-gradient locations, rocks were turned over and the benthos were collected with plastic forceps. The invertebrates were rinsed with site water to remove debris, but they were not allowed time to depurate. Without depuration, the metal concentrations in the invertebrates represent the ''dose'' of metals received by fish.

Fish Sampling Procedure

Fish were collected by electrofishing sections of the river. Different species of fish reside in various reaches of the CDA River, so it was impossible to collect the same species of fish throughout the river. Whole perch (*Perca flavescens*) were collected from five low-gradient sites: LCDR1 (25 ± 3 g), LCDR2 (23 ± 2 g), LCDR3 (23 ± 3 g), HSR (18 ± 9 g), and SJ (53 ± 11 g). Brook trout (*Salvelinas fontanelis*) were collected from NF (189 ± 49 g) and PH (207 ± 24 g), and cutthroat (*Oncorhyncus* sp.; 471 ± 230 g) and rainbow trout (*Oncorhyncus clarki;* 266 ± 216 g) were collected from CT. Metals were measured in gill and kidney. Tissues from NF brook trout were a reference for PH, and SJ perch were a reference for the LCDR and HSR sites. But no reference samples were available for the CT cutthroat and rainbow trout.

Metal Analyses

Sediment, biofilm, and invertebrate samples were processed and analyzed for As, Cd, Cu, Hg, Pb, and Zn at the Environmental and Contaminants Research Center, Columbia, MO. All samples were lyophilized and subjected to a nitric, hydrochloric, and hydrogen peroxide acid digestion procedure conducted in sealed CEM® teflon vessels in a microwave oven. Digestates were analyzed for Cd and Pb by graphite furnace atomic absorption spectroscopy (AAS) with Zeeman background correction, and for Cu and Zn by flame AAS. Arsenic was measured by flow injection–hydride generation AAS in sediments and by furnace AAS in biofilm and invertebrates. Concentrations were determined by calculation from a standard line for all elements except As in biofilm and invertebrates, which was determined with the method of standard additions.

Fish tissues were processed and analyzed for As, Cd, Cu, Pb, and Zn at the Fish Physiology and Toxicology Laboratory, University of Wyoming, Laramie, WY. Fish tissues were ground in a liquid nitrogen–cooled mortar with a cooled ceramic pestle. The tissues were dried at 58°C for 48 h and digested with 30% nitric acid at 76°C for 48 h. The fish tissues were analyzed for As, Cd, Cu, and Pb by graphite furnace AAS with Zeeman background correction and for Zn with flame AAS.

Quality control was monitored for all chemical analyses. Instrument calibration was verified by analyzing certified calibration solutions during each instrumental run. These external reference standards were generally within 80% to 120% of the nominal concentrations. All of the sample spikes for sediments, biofilm, and invertebrates were within 80% to 120% recovery. And the percent recovery of sample spikes for the fish matrix ranged from 50% to 150%. Preparation blanks were prepared to detect potential contamination during the digestion procedure. These preparation blanks generally measured below the detection limit.

Statistics

Concentrations of metals measured in sediment, biofilm, invertebrates, invertebrates from functional feeding groups, and whole fish collected from the test sites were compared to concentrations in samples collected from the reference sites. Because a baseline of data had not been established for sediment, biofilm, and invertebrates in the Coeur d'Alene Basin prior to 1994, data from multiple reference sites were combined to define a baseline value (REF). This method of establishing

a baseline has been used previously when data from prior years were not available for comparisons (Farag *et al.* 1995). Multiple reference sites were not sampled for fish and the functional feeding group study. Data for all sites were tested for homogeneity of variances and transformed when necessary. Under the assumption of equal variances, Dunnett's procedure (Dunnett 1955) was used to make one-tailed t test comparisons between each test site and the REF for each metal. Invertebrates of different functional feeding groups were compared using Tukey's test. When the data failed to meet the homogeneity of variances assumption ($p \leq 0.05$) or if the data contained values below the detection limit, the nonparametric Kruskal-Wallis test (Kruskal and Wallis 1952) was performed to test for differences between means. If any difference was detected, means comparisons between each test site and REF were made using nonparametric methods as described by Zar (1984).

Concentrations of metals measured in gill and kidney tissues from brook trout and for different size invertebrates were compared using t tests between NF and PH for gill and kidney, and between the small and large invertebrates for each metal. When the variances were unequal ($p \leq 0.05$), an approximate t statistic was calculated for the t test using Satterthwaite's approximation for the degrees of freedom (Satterthwaite 1946). If the data contained values below the detection limit, a nonparametric analysis was performed using the Mann-Whitney U test (Mann and Whitney 1947). All data were analyzed with the SAS statistical software package (SAS 1989).

Results

Pathway

In general, the ranking of food-web components from largest to smallest concentrations of metals (µg/g dry wt) were as follows: biofilm ≅ sediments > invertebrates > whole fish (Tables 1–4). Concentrations of most metals were generally the greatest in samples collected from the NM or CC sites (*e.g.*, biofilm > 12,000 µg Pb/g) and have the following general trend: NM > CC > CT > PH > LCDR1 > LCDR3 = HSR > PC, LCDR2, and SR.

The pattern of metal accumulation was similar between sediment and biofilm (Tables 1 and 2). The concentrations of Cd, Pb, and Zn in the sediments and biofilm were greater, often 50 to 100 times greater, in samples from most test sites when compared to the REF ($p \leq 0.05$). The concentrations of Cd and Zn in sediments and biofilm were greater than the REF as far downstream as SR, which is located approximately 80 km from PH. Arsenic, Cu, and Hg were also elevated in samples from many of the test sites. A difference between sediment and biofilm accumulation was that biofilm accumulated the greatest concentrations of Zn (>80,000 µg Zn/g) at CT as opposed to the CC and NM sites (26,450 and 27,200 µg Zn/g respectively).

The concentrations of Pb and Zn were greater in invertebrates from most of the test sites compared to the REF (Table 3). These high concentrations of metals persisted in invertebrates downstream from NM and CC. For example, invertebrates collected at LCDR1 contained >3,000 µg Zn/g, which was 10 times greater than the 324 µg Zn/g in invertebrates from the REF. Also, invertebrates from the site furthest downstream, SR, had three times the amount of Pb measured in invertebrates from the REF.

The concentrations of Pb in whole perch were much less than that measured in sediments, biofilm, and invertebrates. However, whole fish had elevated Zn at LCDR1, LCDR2, LCDR3, and HSR; and had elevated Cd, Pb, and Hg at LCDR3 and HSR (Table 4) when compared to the REF (SJ). Arsenic generally measured below the detection limit in whole fish (data are not presented).

Functional Feeding Group and Size

Concentrations of metals differed among functional feeding groups of benthic macroinvertebrates at CT (Table 5). For all metals except Cu, shredders-scrapers had the largest concentrations of metals, whereas engulfers had the smallest concentrations of metals. Rankings of functional feeding groups for concentrations of As, Cd, Pb, and Zn (from largest to smallest) were as follows: shredders-scrapers (*Pterenarcella* and *Pteronarcys*) > shredders-gatherers (*Tipula*) > filterers (*Arctopsyche*) > engulfers (*Calineuria*). For As, Cd, Hg, and Zn, the shredders-scrapers had significantly greater concentrations of metals than the other functional feeding groups. Engulfers had a significantly smaller concentration of Pb than the other functional feeding groups, and Hg and Zn concentrations did not differ significantly among engulfers, filterers, and shredders-gatherers.

Concentrations of Cu in invertebrates followed a slightly different pattern, wherein engulfers had some of the highest concentrations of Cu in their tissues. The ranking of functional feeding groups for concentrations of Cu (from largest to smallest) was as follows: shredders-scrapers and engulfers > shredders-gatherers > filterers. The shredders-scrapers and engulfers had a greater concentration of Cu than the shredders-gatherers and the filterers.

Within a functional feeding group, the size of invertebrates appeared to influence the concentrations of metals at PH. Small filterers, *Simulium*, had greater concentrations of the metals than the large filterers, *Arctopsyche* (Table 5). Concentrations were significantly different for all metals except Pb, where the variability of data for small filterers was large. But the magnitude of difference was the greatest for concentrations of Pb where *Simulium* from PH had 794 µg Pb/g—five times greater than the 156 µg Pb/g measured in the tissues of *Arctopsyche*.

Specific Fish Tissues

Kidneys of brook trout from PH and cutthroat trout from CT had Pb concentrations of 128 µg/g and 96 µg/g, respectively, which were 640 and 480 times higher than the 0.2 µg Pb/g measured in the kidneys of brook trout from REF (Table 6). Gills of brook trout collected from PH were 255 times higher in Cd than gills from reference fish. The mean concentrations of Cd, Pb, and Zn in kidneys and gills were significantly greater in brook trout collected from PH as compared to fish tissues from the REF. Therefore, while the absolute concentrations of metals were the greatest in sediment and biofilm, the ratio of metals concentrations in test and reference was the greatest in the kidneys and gills of fish. The concentrations of metals in kidneys and gills of fish from CT were generally similar to the concentrations of metals measured in brook trout from PH (Table 6). The data from cutthroat trout and rainbow trout are presented for comparison, but no statistical inferences were made with these data.

Table 1. Metal concentrations in sediments from the Coeur d'Alene and Spokane Rivers, Idaho, 1994. Values are mean (standard error of the mean)

		Metal Concentration (µg/g dry weight)					
Site	n	As	Cd	Cu	Hg	Pb	Zn
NF	4	5.6 (1.3)	0.3 (0.0)	13 (2)	0.06 (0.03)	57 (18)	130 (16)
SJ	4	2.4 (0.1)	0.2 (0.0)	23 (2)	<0.06 (0.00)	10 (0)	61 (3)
ML	4	13.1 (2.3)	1.4 (0.1)	47 (18)	0.13 (0.01)	203 (16)	827 (478)
REF[a]	12	7.0 (1.6)	0.6 (0.2)	27 (7)	0.08 (0.01)	90 (26)	339 (178)
CC	4	91.8 (13.1)*	49.3 (6.5)*	281 (11)*	5.48 (0.67)*	9,187 (522)*	8,543 (931)*
NM	4	8.3 (5.2)	106.5 (33.3)*	170 (18)*	6.23 (2.93)*	4,503 (25)*	19,700 (4,699)*
PC	4	17.5 (3.8)	2.3 (0.8)	24 (5)	0.06 (0.02)	264 (79)	469 (139)
PH	4	179.0 (11.1)*	83.0 (24.9)*	170 (3)*	3.78 (0.30)*	4,757 (295)*	8,130 (2,538)*
CT	4	107.5 (7.0)*	14.5 (2.4)*	77 (8)*	1.78 (0.38)	2,390 (138)*	2,543 (108)*
LCDR1	4	137.5 (12.2)*	33.0 (2.7)*	154 (17)*	3.33 (0.03)*	6,810 (1,469)*	6,790 (858)*
LCDR2	4	57.8 (5.6)*	24.8 (4.2)*	71 (7)*	2.05 (0.21)	2,175 (293)*	3,290 (333)*
LCDR3	4	132.3 (15.0)*	27.0 (2.7)*	101 (8)*	3.23 (0.23)*	3,850 (442)*	4,475 (474)*
HSR	4	170.5 (17.9)*	25.5 (1.9)*	89 (8)*	2.78 (0.14)	3,363 (267)*	3,895 (276)*
SR	4	8.7 (1.9)	6.7 (4.5)*	18 (9)	0.09 (0.06)	253 (153)	1,197 (459)*

[a] REF = pooled value for NF, SJ, and ML sites

* Indicates that a concentration is significantly greater than pooled reference (REF), using the following tests: As, Cd, Cu, Pb, Zn—Dunnett's one-tailed t test, ($p \leq 0.01$); Hg—nonparametric comparisons of REF to other groups ($p \leq 0.05$)

Table 2. Metal concentrations in biofilm from the Coeur d'Alene and Spokane Rivers, Idaho, 1994. Values are mean (standard error of the mean)

		Metal Concentration (µg/g dry weight)					
Site	n	As	Cd	Cu	Hg	Pb	Zn
NF	4	9.6 (0.6)	2.0 (0.5)	9 (1)	0.04 (0.01)	38 (15)	450 (102)
SJ	4	7.2 (2.3)	0.1 (0.1)	25 (6)	<0.05 (0.00)	18 (2)	67 (7)
ML	4	27.3 (0.5)	1.8 (0.1)	21 (1)	0.13 (0.01)	279 (15)	511 (26)
REF[a]	12	14.7 (2.8)	1.3 (0.3)	18 (3)	0.07 (0.01)	112 (36)	343 (67)
CC	4	101.3 (7.1)*	150.3 (13.5)*	299 (11)*	3.35 (0.10)*	12,550 (612)*	26,450 (1,880)*
NM	4	12.5 (0.6)	69.5 (2.9)*	1,175 (32)*	4.73 (0.09)*	26,425 (923)*	27,200 (1,530)*
PC	4	9.5 (1.0)	3.9 (0.5)*	13 (1)	0.11 (0.04)	333 (98)	1,038 (190)
PH	4	98.8 (11.8)*	179.0 (34.5)*	65 (7)*	1.24 (0.24)	2,015 (282)*	11,578 (933)*
CT	4	155.8 (17.1)*	884.0 (155.3)*	130 (21)*	1.12 (0.17)	3,818 (384)*	83,300 (11,457)*
LCDR1	4	126.8 (5.1)*	27.5 (1.9)*	104 (8)*	3.63 (0.40)*	4,803 (515)*	5,400 (228)*
LCDR2	4	15.3 (3.2)	7.0 (1.9)*	18 (3)	0.42 (0.09)	446 (128)*	1,635 (443)*
LCDR3	4	83.5 (17.3)*	20.3 (3.8)*	71 (12)*	1.74 (0.34)*	3,035 (757)*	4,658 (904)*
HSR	4	148.8 (37.9)*	20.8 (2.5)*	86 (5)*	2.18 (0.09)*	3,460 (384)*	4,543 (458)*
SR	4	7.5 (0.6)	15.3 (1.9)*	12 (1)	0.06 (0.02)	169 (35)	2,263 (174)*

[a] REF = pooled value for NF, SJ, and ML sites

* Indicates that a concentration is significantly greater than pooled reference (REF), using the following tests: As, Cd, Cu, Pb, Zn—Dunnett's one-tailed t test, ($p \leq 0.01$) [for Pb and Zn at LCDR2, $p \leq 0.05$]; Hg—nonparametric comparisons of REF to other groups ($p \leq 0.01$) [for LCDR3, $p \leq 0.05$]

Discussion

Metals persist in various components in the CDA River and are biologically available. Canyon creek and NM are two important sources of metals in the CDA Basin. And metals originating from these sites are detected in biota as far downstream as SR.

The concentrations of metals observed in the sediments may be sufficiently large to cause toxicity to invertebrates in the CDA River. Sediment no effect concentrations (NECs) for *Hyalella azteca* (Ingersoll *et al.* 1996) are 100 µg As/g, 8 µg Cd/g, 130 µg Pb/g, and 1,300 µg Zn/g. The sediments exceeded the NEC for Cd and Zn at all but the REF, PC, and SR sites; and the NEC for Pb was exceeded at all but the REF. The NEC for As was exceeded in sediments from five sites on the CDA River (PH, CT, LCDR1, LCDR3, and HSR). Therefore, laboratory assays predict that metals present in concentrations measured in the CDA River may be toxic to benthic macroinvertebrates in the field.

The concentrations of metals in biofilm were about equal to those measured in the sediments of the CDA Basin. Because metals are associated with both the abiotic and biotic components of biofilm (Newman *et al.* 1983, 1985), the large concentrations of metals in this component suggest an important link in the movement of metals up the food chain. Not only

Table 3. Metal concentrations in benthic macroinvertebrates from the Coeur d'Alene and Spokane Rivers, Idaho, 1994. Values are mean (standard error of the mean)

		Metal Concentration (µg/g dry weight)					
Site	n	As	Cd	Cu	Hg	Pb	Zn
NF	4	2.4 (0.6)	1.2 (0.4)	23 (3)	0.06 (0.00)	9 (2)	255 (33)
ML	4	2.1 (0.2)	2.5 (0.2)	17 (1)	0.12 (0.02)	12 (1)	393 (35)
REF[a]	8	2.3 (0.3)	1.9 (0.3)	20 (2)	0.09 (0.01)	10 (1)	324 (34)
CC	4	4.0 (0.3)*	10.4 (0.7)	41 (2)	0.14 (0.02)	661 (69)*	438 (198)
NM	4	2.2 (0.5)	15.2 (3.4)*	201 (59)*	0.52 (0.16)*	3,893 (1,405)*	2,719 (919)*
PC	4	2.3 (0.4)	10.3 (2.4)	35 (6)	<0.10 (0.00)	34 (10)*	1,440 (351)*
PH	4	41.8 (1.0)*	52.3 (1.0)*	72 (5)*	0.77 (0.08)*	841 (53)*	2,658 (71)*
CT	4	9.0 (2.9)*	16.7 (3.7)*	34 (3)	0.19 (0.04)	292 (90)*	1,735 (273)*
LCDR1	4	47.8 (6.7)*	34.8 (1.0)*	94 (5)*	2.08 (0.10)*	2,335 (77)*	3,050 (127)*
LCDR2	4	2.2 (0.2)	8.4 (1.6)	28 (3)	0.18 (0.01)	46 (4)*	386 (96)
LCDR3	1	97.0	57.0	90	12.0	3,900	2,350
HSR	1	18.0	10.0	50	0.12	335	746
SR	4	2.3 (0.2)	3.0 (0.3)	16 (1)	<0.10 (0.00)	32 (4)*	555 (15)

[a] REF = pooled value for NF and ML sites

* Indicates a concentration is significantly greater than pooled reference (REF), using the following tests: As, Pb, Zn—Dunnett's one-tailed t test [Pb and Zn, $p \leq 0.01$; As, $p \leq 0.05$]; Cd, Cu, Hg—nonparametric comparisons of REF to other groups ($p \leq 0.01$) [for Cd at NM, $p \leq 0.05$]

Table 4. Metal concentrations in whole juvenile perch (*Perca flavescens*) from the Coeur d'Alene and St. Joe Rivers, Idaho, 1994

		Metal Concentration (µg/g dry weight)				
Site	n	Cd	Cu	Hg	Pb	Zn
REF (SJ)	5	0.1 (0.0)	7.8 (0.4)	0.21 (0.01)	<0.3 (0.0)	89 (5)
LCDR1	5	0.4 (0.1)	8.2 (0.4)	0.30 (0.02)	14.6 (1.6)	141 (7)*
LCDR2	5	0.6 (0.1)	9.6 (0.9)	0.33 (0.03)	15.1 (2.6)	144 (11)*
LCDR3	5	1.1 (0.2)*	7.9 (0.3)	0.50 (0.04)*	45.0 (9.4)*	185 (20)*
HSR	4	1.5 (0.3)*	9.3 (0.2)	0.62 (0.12)*	55.2 (8.9)*	252 (40)*

Values are mean (standard error of the mean). Note: Results for LCDR3 and HSR were not included in the statistical analyses, because n = 1.

* Indicates a concentration is significantly greater than the reference (REF), using the following tests: Cu, Hg, Zn—Dunnett's one-tailed t test ($p \leq 0.01$); Cd, Pb—nonparametric comparisons of a control to other groups ($p \leq 0.01$)

are biofilm samples important to trace the pathway of metals, but of all components collected, biofilm samples were the most consistent in availability among sites and were collected with the most ease and efficiency.

Metals present in invertebrate tissues document one route through which metals move further up the food chain. However, the functional feeding group of invertebrates may influence the concentrations of metals accumulated in benthic macroinvertebrates. Thus, one must be careful in making specific comparisons of metal concentrations between composite invertebrate samples. A composite sample with a larger proportion of scrapers-shredders (herbivores) will have a greater concentration of metals simply because scrapers-shredders accumulate greater concentrations of metals than the other functional feeding groups. Scrapers-shredders may accumulate greater concentrations of metals than other invertebrates because they feed on biofilm and its concentrated metal load. These data are supported by Kiffney and Clements (1993), who documented that invertebrates feeding on biofilm had greater concentrations of metals than representatives of other functional feeding groups.

The concentrations of metals in benthic macroinvertebrates from similar functional feeding groups can be compared between drainages contaminated with metals. The concentrations of Cd and Zn in the filter feeders (*Arctopsyche*) from CT were 8.1 and 1,007 µg/g, respectively, compared to 4.5 and 600 µg/g in the tissues of *Arctopsyche* from a metals contaminated site on the Arkansas River, sampled during the summer season (Kiffney and Clements 1993). However, *Arctopsyche* from the Arkansas River had greater concentrations of Cu than those from CT (30 µg/g vs. 18 µg/g). Therefore, the type of metal load in invertebrates is different between these two drainages.

For invertebrate biomonitoring purposes, interbasin comparisons of metal distribution should be performed with invertebrates of similar size and functional feeding type. But important data also result from measurements of composite samples. Composite invertebrate samples represent the invertebrate community as it is available to predatory fish. Also, in the field benthic invertebrates are available to fish without first being allowed to depurate in freshwater. Therefore, the concentrations of metals in composite invertebrate samples, where inverte-

Table 5. Metal concentrations in benthic macroinvertebrates that represent various functional feeding groups and sizes from the CDA River, Idaho, 1994

	Metal Concentration (µg/g dry weight)					
Site	As	Cd	Cu	Hg	Pb	Zn
CT						
Herbivore (shred/scrape)	15 (2.5)[a]	22 (3.1)[a]	34 (2.1)[a]	0.29 (0.03)[a]	478 (131)[a]	2,630 (184)[a]
Omnivore (shred/gather)	7 (1.2)[b]	12 (0.6)[b]	23 (0.3)[b]	0.15 (0.02)[b]	206 (17)[a]	1,263 (37)[b]
Detritivore (filter)	5 (0.5)[b,c]	8 (0.6)[b,c]	18 (0.4)[c]	0.15 (0.01)[b]	177 (16)[a]	1,007 (53)[b]
Carnivore (engulf)	3 (0.3)[c]	6 (0.5)[c]	32 (1.4)[a]	0.11 (0.01)[b]	74 (12)[b]	973 (34)[b]
PH						
Large (filter)	9 (0.8)[a]	12 (0.3)[a]	20 (0.5)[a]	0.17 (0.02)[a]	156 (7)[a]	1018 (38)[a]
Small (filter)	19 (1.7)[b]	25 (0.8)[b]	36 (2.2)[b]	0.39 (0.04)[b]	794 (330)[a]	1658 (132)[b]

Concentrations are mean (standard error of the mean) and n = 4
Values within a site with the same letter are not significantly different ($p \leq 0.01$)

Table 6. Metal concentrations in tissue of fish from the Coeur d'Alene River, Idaho, 1994. Values are mean (standard error of the mean)

			Metal Concentration (µg/g dry weight)			
Site	Species	n	Cd	Cu	Pb	Zn
Kidney						
REF (NF)	Brook	4	3.1 (1.5)	6.6 (0.3)	0.2 (0.0)	132 (1)
PH	Brook	6	233.6 (37.8)*	77.1 (16.8)*	127.7 (43.5)*	499 (48)*
CT	Cutthroat	4	155.2 (20.5)	113.4 (25.7)	96.4 (48.7)	296 (40)
CT	Rainbow	6	119.5 (22.0)	104.1 (17.2)	102.7 (41.2)	440 (39)
Gill						
REF (NF)	Brook	4	0.5 (0.1)	5.9 (0.8)	1.6 (0.7)	123 (22)
PH	Brook	6	127.4 (40.8)*	30.6 (10.8)	24.8 (6.9)*	594 (84)*
CT	Cutthroat	4	46.8 (10.9)	16.4 (1.6)	78.8 (28.0)	806 (204)
CT	Rainbow	6	108.7 (11.1)	15.3 (2.0)	18.4 (2.9)	1,233 (210)

* Indicates that SF concentration is significantly greater than the NF reference, using the following tests: Cd, Cu, Pb, Zn—t test ($p \leq 0.05$); Pb in gill tissue—Mann-Whitney U test ($p \leq 0.05$)
Note: data from cutthroat and rainbow trout were not included in the statistical analyses

brates have not been depurated, represent the potential invertebrate ''dose'' of metals available to fish in the CDA Basin.

The Clark Fork River, Montana is another river in the western United States where historical mining practices have resulted in elevated concentrations of metals in biota (Farag *et al.* 1995). We can compare the potential dose of metals to fish in the Clark Fork River to that in the CDA River because composite invertebrate samples were collected from sites with elevated metals in both drainages. The concentration of Pb in invertebrates was 29 times greater in composite samples collected from CT (292 µg/g) compared to invertebrates collected from the Clark Fork River, near Warm Springs (<10 µg/g) (Poulton *et al.* 1995). The mean concentration of Zn was six times greater in invertebrates from CT (1,735 µg Zn/g) as compared to invertebrates from the Clark Fork River, near Warm Springs (approx. 300 µg Zn/g). However, the Clark Fork invertebrates were more contaminated with Cu than the CDA invertebrates as the mean concentration of Cu (>100 µg Cu/g) was three times greater in invertebrates collected from the Clark Fork River, than in invertebrates from CT (34 µg Cu/g).

One objective of this study was to document the pathway of metals transfer in the CDA River. The concentrations of metals in benthic macroinvertebrates represent one biological link in that pathway. As described above, benthic macroinvertebrates are often collected to document the concentrations of metals in the biota of river systems contaminated with metals. In general, invertebrates from the CDA River accumulated more Pb and Zn but less Cu than invertebrates collected from other river systems where historical mining activities have occurred. This pattern was observed regardless of whether we compared concentrations of metals in invertebrates of the same genus or we compared composite samples.

We have also observed that the size of invertebrates within a functional feeding group may affect the accumulation of metals in invertebrates. Because we compared the concentrations of metals in small *Simulium* with those in large *Arctopsyche*, the effects of differences in taxa on metal accumulation cannot be completely discounted. However, these taxa have similar feeding habits and both feed on filtered organic material. One difference between the two taxa is that large *Arctopsyche* may incidentally filter animal tissue in addition to plant material. The smaller *Simulium* however, is not as likely to filter animal tissue. Sampling identical species may have had little impact on our results because this difference also exists between large and small *Arctopsyche* and can be attributed to size. Furthermore, van Hattum *et al.* (1991, 1996) reported that invertebrate size, rather than species or sex, explained most of the variability in metal accumulation in invertebrates. Our data, together with the available literature (Timmermans *et al.* 1989; van Hattum *et al.* 1991, 1996) leads us to conclude that the differences we

observed in metal concentrations between small *Simulium* and large *Arctopsyche*, are predominantly due to size.

Because the concentrations of metals are inversely proportional to size, early-lifestage and juvenile fish are more at risk than older age–class fish for two reasons: 1) young salmonids (<100 mm long) feed exclusively on insects and zooplankton; and 2) they feed on the smallest sizes of invertebrates (Bjornn and Likens 1986). Adult fish that feed on fish and larger invertebrates will receive a smaller dose of metals than young fish. Woodward *et al*. (1994, 1995) documented that invertebrates from the Clark Fork River (Montana) contaminated with metals significantly affected the growth and other physiological parameters in early-lifestage fish. The diets in the Woodward *et al*. studies were prepared by collecting composites of large and small invertebrates from the field and feeding them to fish in the laboratory. In light of our data, those feeding studies may have underestimated the effects of metals via the diet on early-lifestage fish.

While the concentrations of metals were the greatest in sediment and biofilm samples collected from the CDA Basin, the greatest magnitude of differences between test and reference sites existed in the gills and kidneys of fish. The concentration of metals in surface water contributes to the accumulation of metals in the gills and kidneys of fish (Farag *et al.* 1994). Gills are exposed to metals via water because they are constantly in direct contact with the surrounding water. Kidneys are exposed to metals via water because blood flows from gills into the common carotid artery, which supplies the kidney (Farrell 1993). From 1992 through 1994, concentrations of total recoverable Cd, Pb, and Zn in the water from test sites were greater than in water from a reference site (Table 7). Thus, elevated concentrations of metals have been historically documented in the CDA River (Ellis 1940; Hornig *et al*. 1988) and during the time period of this study. These elevated concentrations of metals have likely contributed to the accumulation of metals in the gills and kidneys of fish in the CDA River.

The concentrations of metals measured in whole fish collected from the CDA River greatly exceed the average concentrations of metals in fish from throughout the United States. Over 300 whole fish were collected from over 100 stations across the country between 1976 and 1984 (Lowe *et al.* 1985; Schmitt and Brumbaugh 1990). The 85th percentiles (values for which 85% of samples are below) of metals measured in those samples were 0.33 µg Cd/g, 1.31 µg Pb/g, and 201 µg Zn/g (80% moisture assumed to calculate µg/g dry wt), but were considerably less than comparable measurements from our study: 1.5 µg Cd/g, 55 µg Pb/g, and 252 µg Zn/g in fish from HSR.

In summary, large concentrations of metals enter the CDA Basin where mining activities occurred, and the metals are transported downstream. The metals move from water and sediments to biofilm, invertebrates, and fish throughout the basin. Although the absolute concentrations of metals are the highest in biofilm and sediments, the metals have unquestionably bioaccumulated in fish. These data document that the metals in the CDA Basin are bioavailable. Although they do not biomagnify between trophic levels, they do bioaccumulate. Metals may bioaccumulate to concentrations that cause physiological effects in indigenous fish (Farag *et al.* 1995). Also, exposure of early-lifestage fish to metals may be greater than in adults because smaller invertebrates accumulate more metals than larger invertebrates, and trout less than 100 mm long feed exclusively on small invertebrates (Bjornn and Likens 1986).

Table 7. Metal concentrations in surface water from the Coeur d'Alene River Idaho, October 1992 through September 1994

	Metal Concentration (total recoverable µg/L)							
Site	Cd	n	Cu	n	Pb	n	Zn	n
REF (NF)	1 (0)	21	3 (0)	20	3 (1)	21	20 (3)	21
PH	11 (1)*	22	3 (1)	20	39 (9)*	22	1,675 (145)*	22
CT	3 (0)*	22	3 (0)	20	13 (3)*	22	426 (48)*	22
LCDR2	2 (0)	7	1 (0)	7	31 (11)*	7	358 (54)*	7
HSR	2 (0)*	23	5 (1)	23	38 (7)*	23	382 (27)*	23

Values are mean (standard error of the mean)
* Indicates a concentration is significantly greater than the reference (REF) using the following tests: Cd, Cu, Pb, Zn: nonparametric comparisons of REF to other groups ($p \leq 0.025$); LCDR2: observations only from March through September, 1994
Note: Data taken from USGS Water Resources Data, Idaho (Brennan *et al.* 1994; Harenberg *et al.* 1994)

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